IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Douglas F. Covey
Serial No. 10/008,567
Filed November 5, 2001
Confirmation No. 6682
Examiner Evelyn Mei Huang

Art Unit 1625

11-8
Vcp
9-17-3

For CYTOPROTECTIVE POLYCYCLIC COMPOUNDS

September 5, 2003

DECLARATION OF DOUGLAS F. COVEY

TO THE ASSISTANT COMMISSIONER FOR PATENTS,
Washington, D.C. 20231

I, Douglas F. Covey declare as follows:

1. I am a professor employed by Washington University of St. Louis, located in the State of Missouri.

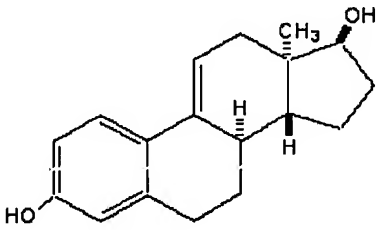
2. I am the inventor of the subject matter claimed in the above-identified U.S. Patent application, Serial No. 10/008,567.

3. I conceived and reduced to practice the invention of claim 36, as presented in Amendment B submitted herewith, prior to October 19, 2000.

4. All work referred to herein was carried out in the United States.

5. Evidence of my conception and reduction to practice of a compound having cytoprotective activity, as described by claim 36 of Amendment B submitted herewith, is attached hereto as Exhibits A, B and C. On information and belief, Exhibit A is a true and

correct copy of pages of a laboratory notebook (with dates deleted) maintained by Zu Yun Cai who, at the time the work described in these pages was performed, was working under my direction and supervision. The work described on these pages was carried out prior to October 19, 2000. Exhibit A identifies and describes the preparation of the following cytoprotective compound:



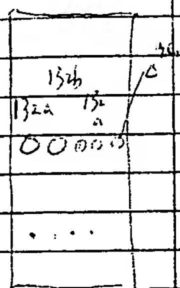
Additionally, on information and belief, Exhibits B and C are true and correct copies of NMR and Ultraviolet spectra, respectively, of the above-referenced compound (with dates deleted) maintained by Zu Yun Cai who, at the time these analyses were performed and the spectra were generated, was working under my direction and supervision. These analyses were performed and the spectra were generated prior to October 19, 2000.

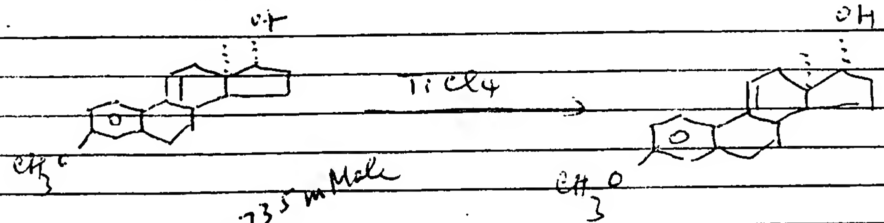
Accordingly, Exhibits A, B and C evidence my conception and reduction to practice of the cytoprotective compounds of claim 36, as amended, prior to October 19, 2000.

6. I declare that all statements made herein of my knowledge are true; and further that these statements were made with the knowledge that willfully making false statements is punishable by fine, imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sept. 5, 2003
Date

Douglas F. Covey





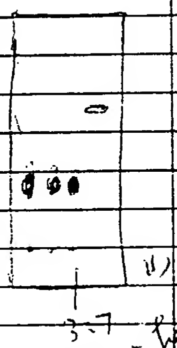
0.735 mMole

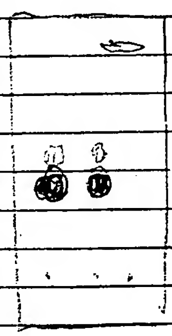
0.25 g ($\Delta^{9(11)}$ separate from $\Delta^{8(9)}$ comp, 0.15 g 20132a, + 0.05 g 20132 b)

+ 10 ml anhydrous CH_2Cl_2 cool to 0° → −10°C (outside NaCl) + 0.75 ml $TiCl_4$ quickly. The reaction mixture became dark brown. Start to end total time 20 min. Temp (outside −0° → −10°C). Take TLC. The reaction is done (1)

Then at 0 → −10°C add 2 ml H_2O. The dark brown color fade to colorless. Then extract c̄ CH_2Cl_2 several times. & washed c̄ brine three times until the aqueous appear almost neutral. dried c̄ Na_2SO_4. Remove the solvent after got crude 0.24 run NMR 20138 OK.

After work up. Take TLC as 2





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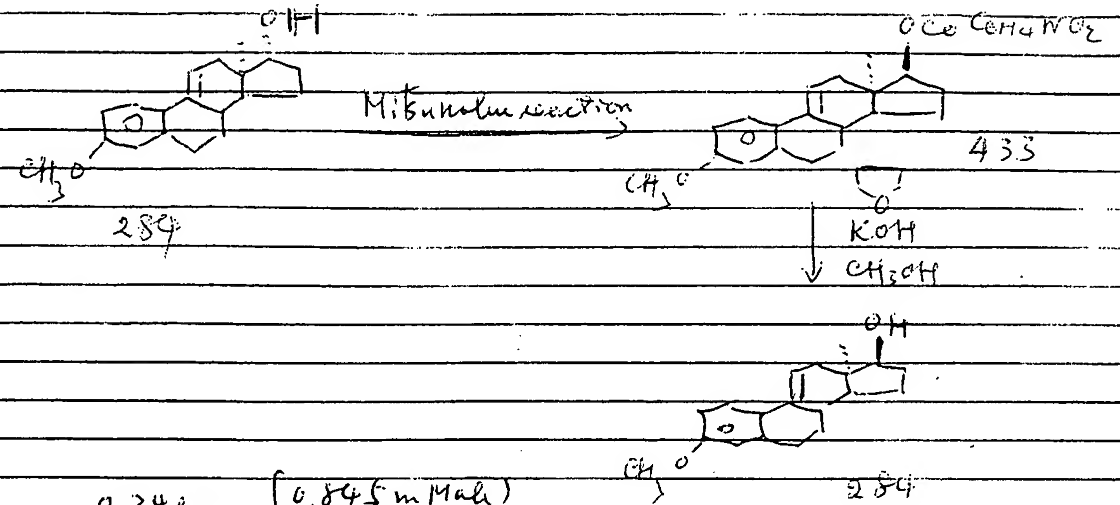
SIGNATURE ______________________ DATE ________ 19 ____

READ AND UNDERSTOOD ______________________ DATE ________ 19 ____



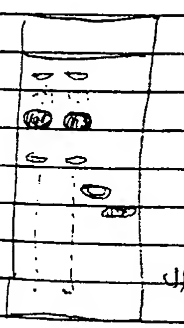
0.24 gm. (0.845 m Mole)
ST ~~0.25~~ gm (~~0.735~~ m Mole)

$C_2H_5OOCN=NCOOC_2H_5$ 0.735 × ~~1.3~~ 2.5 = ~~0.956~~ 1.84 m Mole (×174) = ~~166.32~~ 320 mg

$NO_2C_6H_4COOH$ 0.735 × 2.5 = ~~1.5~~ 1.84 (×167) = 306.86

Ph_3P 0.735 × ~~1.3~~ 2.5 = ~~0.956~~ 1.84 (× 262.29) = ~~250.62~~ 481.95 mg

ST 0.24 gm + 7 ml anhydrous toluene + $NO_2C_6H_4COOH$ 0.308 g
+ Ph_3P 0.49 gm. + ~~[illegible]~~ Dead 0.45 g ~~heat out side bath.~~
80–90°C Start 11:00 AM. — 5:00 PM. (Stop)
Take TLC

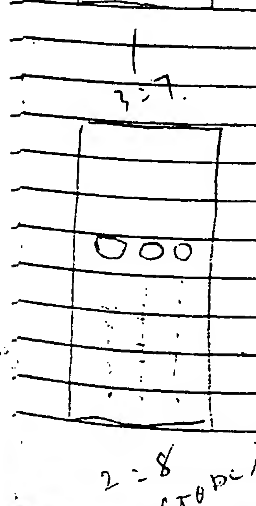


Remove the solvent as much as possible, ~~then~~ almost dry
Then Run flash chromatography, 7%, 10%, 15%, 30%, 60%
compd came down from 10%

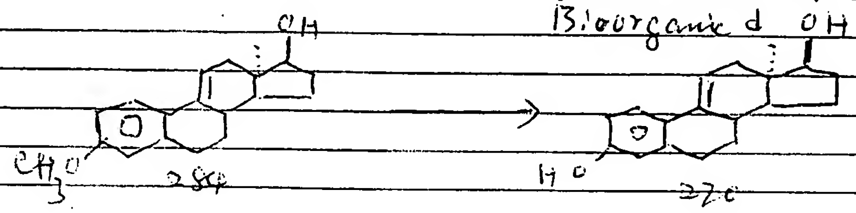
Got 150 mg not very accurate yellow solid NMR 2013.9 UK

$\frac{240}{284} \times 433 = 365.9$

$\frac{150}{365.9} = 41\%$



Ref. J Org. Chem. 1987, 52, 4235-4238
J. Biol. Chem. 187, 1950, 557, 562, 563
Bioorganic & Medicinal Chemistry Letter Vol. 7 1995;



To under N_2 1.5M DIBAL 1.5ml DIBAL = 2.25 mMole was added
dry 60mg (0.21 mMole) in 3ml toluene solution. stir & heat
outside bath 120°C 11:30 - overnight.
Temperature keep 125°C (outside oil bath)

Take TLC its done. add ice to the bottle,
acidify 3N 3ml to PH as 4. extract c EtOAc
several times washed c brine dried c Na_2SO_4
to get 70mg.
Flash Chromatography using 10% EtOAc in hexane 15%, 20%, 30%
got 60mg which crystallization from acetone & hexane
got 30mg 20141 10mg second fraction. 20141m.
mp 239-241°C

$\frac{60}{284} \times 270 = 57$. $\frac{40}{57} = 70\%$

determination of $[\alpha]_D$

20141, 20141, 20125, 3:7 EtOAc in hexane run 3 times.

		dioxane
wt.	-0.348	0.000
~~12.0665~~	-0.347	0.000
12.0612	-0.348	
	.349	
~~0.0053 in 2ml dioxane~~	.348	UV. ~~wet~~ wt
	.347	11.8898
1ml = 0.00265	.347	11.8882
	.348	0.0016 = 1.6 mg
	.346	λmax 263.0 (CH_3OH)
$[\alpha]_D^{25} = \frac{-0.348}{0.00265} = -131.32$ (c = 0.265)	.347	~~1.6mg~~ Abs. 1.7901
	.349	
	.349	1.6mg in 5ml CH_3OH
	.347	take 0.5ml dilute to 5ml

average -0.348

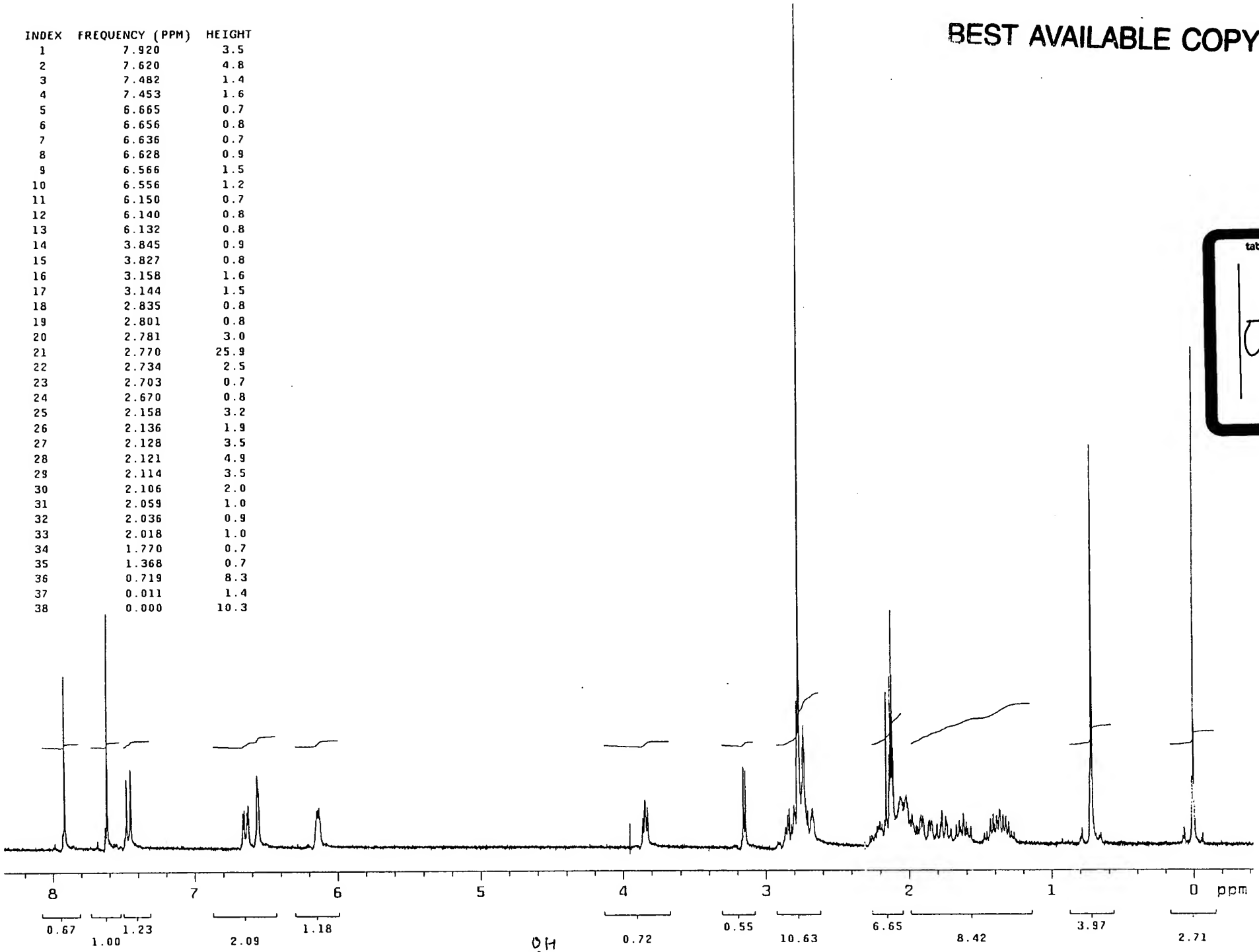
Send Dr Corey 25mg

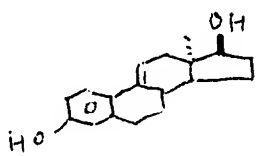
~~ZYC-12~~ 2 ZYC-12

$\varepsilon = \frac{1.7901}{\frac{0.00016 \times 200}{270}} = 15106.329$ *

SIGNATURE 20141 DATE 19
READ AND UNDERSTOOD DATE 19

INDEX	FREQUENCY (PPM)	HEIGHT
1	7.920	3.5
2	7.620	4.8
3	7.482	1.4
4	7.453	1.6
5	6.665	0.7
6	6.656	0.8
7	6.636	0.7
8	6.628	0.9
9	6.566	1.5
10	6.556	1.2
11	6.150	0.7
12	6.140	0.8
13	6.132	0.8
14	3.845	0.9
15	3.827	0.8
16	3.158	1.6
17	3.144	1.5
18	2.835	0.8
19	2.801	0.8
20	2.781	3.0
21	2.770	25.9
22	2.734	2.5
23	2.703	0.7
24	2.670	0.8
25	2.158	3.2
26	2.136	1.9
27	2.128	3.5
28	2.121	4.9
29	2.114	3.5
30	2.106	2.0
31	2.059	1.0
32	2.036	0.9
33	2.018	1.0
34	1.770	0.7
35	1.368	0.7
36	0.719	8.3
37	0.011	1.4
38	0.000	10.3

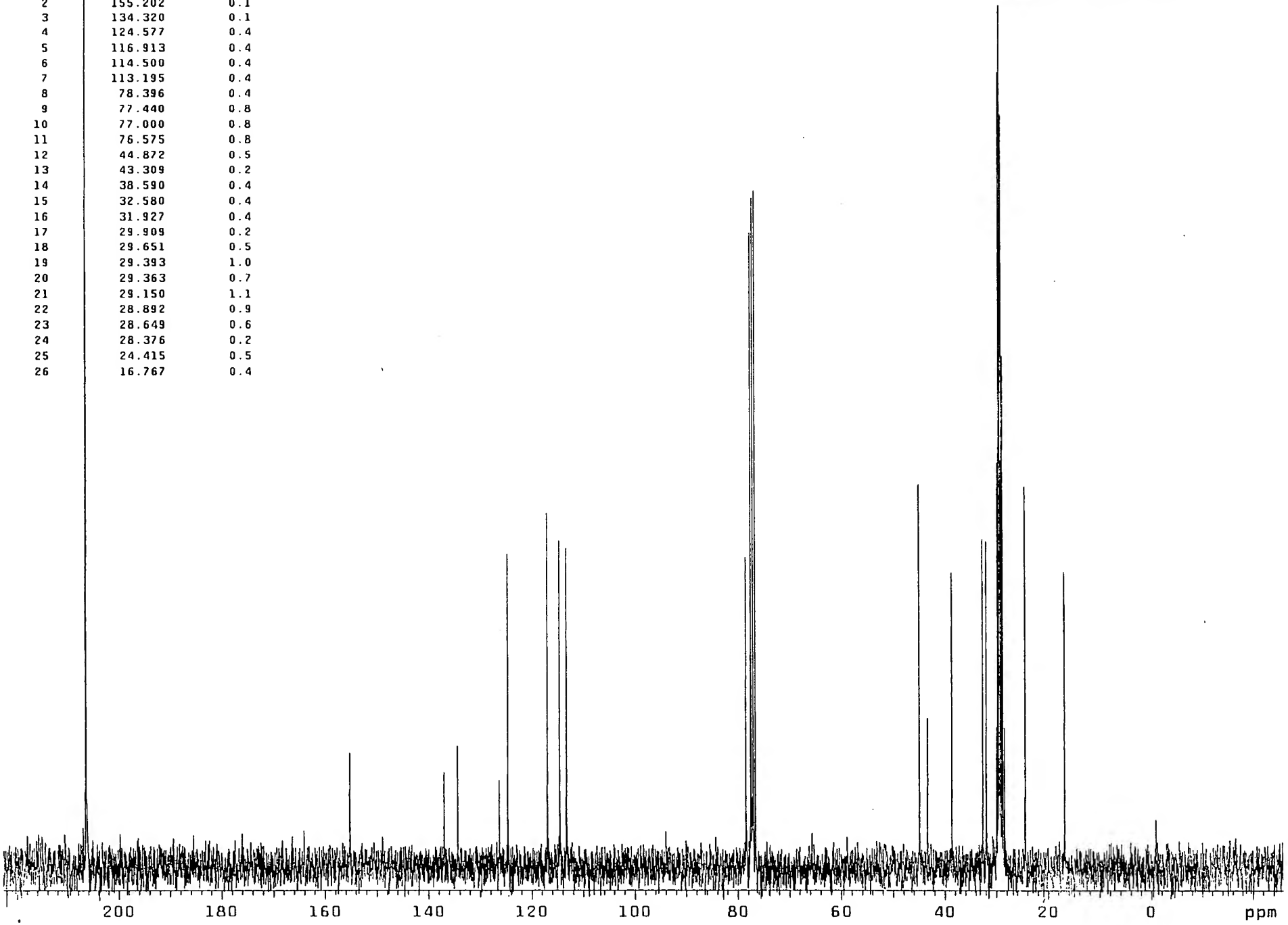




$CDCl_3 : CD_3COCD_3 = 2:1$

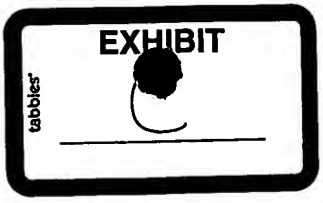
ZYC-12

INDEX	FREQUENCY (PPM)	HEIGHT
1	206.345	1.3
2	155.202	0.1
3	134.320	0.1
4	124.577	0.4
5	116.913	0.4
6	114.500	0.4
7	113.195	0.4
8	78.396	0.4
9	77.440	0.8
10	77.000	0.8
11	76.575	0.8
12	44.872	0.5
13	43.309	0.2
14	38.590	0.4
15	32.580	0.4
16	31.927	0.4
17	29.909	0.2
18	29.651	0.5
19	29.393	1.0
20	29.363	0.7
21	29.150	1.1
22	28.892	0.9
23	28.649	0.6
24	28.376	0.2
25	24.415	0.5
26	16.767	0.4



ZYC-12

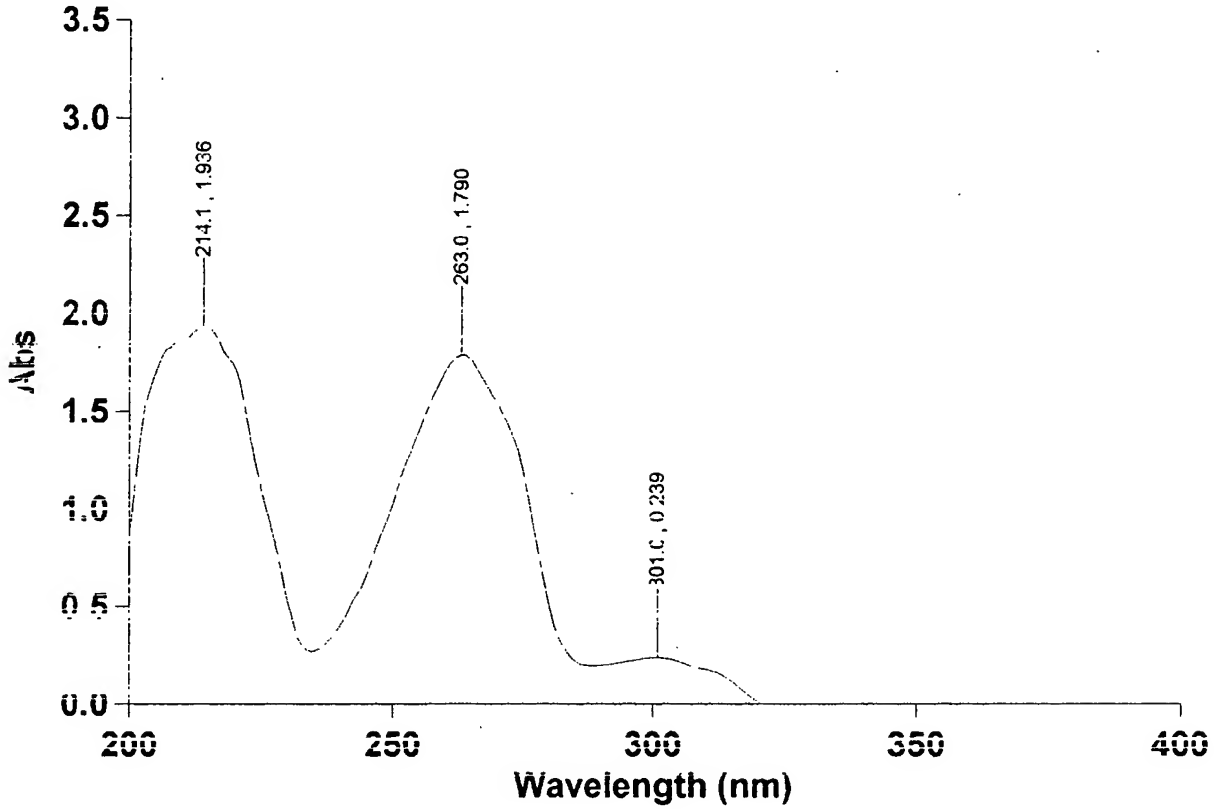
$CDCl_3 : CD_3COCD_3 = 2:1$



1.6 mg in 5 ml CH3OH
Take 0.5 ml dilute to 5 ml.

$$\varepsilon = \frac{1.7901}{\frac{0.00016 \times 200}{270}} = 15106.329$$

Dr Garland Marshall's LAB.
Instrument Serial Number EL98103244



Scan Analysis Report

Report Time :
Batch:
Software version: 02.00(25)
Operator: guest

Sample Name: 20141

Collection Time

24C-12

Peak Table
Peak Style Peaks
Peak Threshold 0.0100
Range 400.0nm to 199.9nm

Wavelength (nm)	Abs
301.0	0.2389
263.0	1.7901
214.1	1.9359